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Green Synthesis of nano calcium oxide using Olive plant extracts and studying its effect on bacteria causing dental caries in children

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ABSTRACT: One hundred clinical samples were collected from children suffering from oral diseases such as tooth decay and root canal inflammation under the supervision of a specialist dentist, using sterile cotton swabs from the site of infection, for patients visiting specialized dental centers in Baiji city, Salah al-Din Governorate. Their ages ranged from 3 to 13 years, and for the period from the beginning of September 2023 until February, where 100 pathogenic bacterial isolates were isolated from clinical samples, diagnosed based on cultural and microscopic characteristics, in addition to diagnosis with the Vitek2 device for diagnosis at the species level. The genus *Streptococcus mutans* appeared in it at a rate of 18.18%, which is the most significant percentage among other genera. The following species were obtained: *Staph. aureus*, *Staph.epidermis*, *Staph.saprophyticus*, *Micro.luteus*, and *B.subtilius*.

The sensitivity of bacterial isolates to antibiotics was tested, and the results showed that they had the highest resistance to cefepime and the least resistance to Gentamycin. Olive leaves were collected and characterized, the aqueous extract was prepared, and its acidity was estimated. The antimicrobial activity was tested using the disk diffusion method. The study showed that the aqueous extract of olive leaves had inhibitory activity against Staphylococcus aureus bacteria with an inhibition diameter of 23 mm. The bio-manufacturing of nano calcium oxide from olive leaf extract was also carried out, and its inhibitory activity on some bacterial isolates was studied. The results showed that the nano solution has activity against the bacterial genus Staphylococcus aureus. In this study, the anti-oxidative activity (ROS%) of the aqueous extract of fig and olive leaves was determined, as olive plants demonstrated more muscular anti-oxidative activity compared to figs, as well as ascorbic acid, which served as a positive control in this experiment.

Keywords: Green Synthesis, nano calcium oxide, Olive plant extracts, Anti-oxidants, Anti-bacterial

1. INTRODUCTION

Dental caries is one of the most common health problems worldwide, in all age groups, and especially in children. Sugar plays a significant role in dental development, especially in the first years of life (1) It is a dynamic and multifactorial disease, but it is preventable and has known contributing factors. It is caused by tooth decay by acid production resulting from carbohydrate metabolism by certain bacteria (2). The oral microbial ecosystem is constantly exposed to foreign exogenous substances (3). Such conditions are determinants of the establishment and persistence of microbes in this environment, making distinct host-microbe relationships dependent on selective pressures. The pioneer microbial colonizers of the oral cavity, such as *Streptococcus mitis*, *Streptococcus sanguis-nis*, *Streptococcus gordonii*, and *Streptococcus salivarius*, display essential characteristics that make them well suited for this specific niche because

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they can selectively attach to predental cells of the tongue and cheek and can outcompete other microbial species (4). Many species of bacteria naturally inhabit the mouth and are called the oral flora. It has been well-studied that the invasion of various bacteria in the oral cavity may cause various bacterial infections. Therefore, it is very necessary to allow early diagnosis of various pathogenic microbes. The identified bacteria and their strength in the oral environment can help to predict the exact progression of various periodontal diseases (5).

Awareness of the rational use of antibiotics can significantly reduce indiscriminate use, decrease bacterial resistance, and mitigate public health risks. This research aims to develop educational strategies that promote the rational use of antibiotics, which effectively contributes to increasing responsible practices and maintaining drug efficacy for societal health benefits. These extracts are used in many fields, including medical, industrial, and agricultural applications (6) plant extracts are described as chemical or green preservatives and biologically active materials (7). Olive leaf extract is a dark brown liquid with a bitter taste. Olive leaf extract is part of natural medicine and has many health benefits. It is traditionally used as an herbal supplement because it contains polyphenol compounds with beneficial properties such as increasing energy levels and lowering blood pressure. In addition to all the benefits mentioned above, olive leaf extract has antimicrobial properties. As with many natural products, the composition of the extract may vary according to different conditions, such as geographical location, cultivar, and plant nutrition. Olive leaves also contain many powerful and important antioxidants that help protect against cancer because these leaves contain polyphenols and flavonoids (8). Green synthesis is a simple process that uses a variety of biological agents, such as plants, bacteria, fungi, algae, and yeast, without hazardous products (9). Nanoparticles have been established as a promising approach to solving many problems (10) due to overcoming current antibiotic resistance mechanisms by disrupting bacterial membranes and inhibiting biofilm formation.

Therefore, NPs have the ability to combat microbes using multiple mechanisms simultaneously (11) and are gaining significant interest because they may fill gaps where antibiotics frequently fail (12) Calcium oxide (CaO) is an important inorganic compound used in various industries (13). Calcium oxide (CaO) is widely used in cosmetics, medicine, waste treatment, destructive adsorbents, and catalysts (14). In dentistry, it is a highly valued material for its biocompatibility (15), as well as an antimicrobial agent, drug delivery agent, and many other biological applications (16). Compared with conventional CaO particles, CaO nanoparticles showed excellent antimicrobial activity and endotoxin inactivation ability (17). It is important to emphasize that several factors influence the antioxidant activity of plant extracts. These factors include the diversity of antioxidants, the climatic conditions prevailing during plant growth, the stage of maturity at harvest, and storage conditions such as temperature and storage duration, moisture content, and pH levels. The quality and polarity of the solvent used in extraction, the methods used to separate the compounds, and the purity of the biologically active substances also play an important role, in addition to the analytical techniques and substrates used (18). This study aimed Isolation and diagnosis of bacteria causing tooth decay in children. Study of the sensitivity of some bacterial isolates under study to 6 antibiotics. Manufacture of aqueous extract of olive leaves. Green manufacture of nano calcium oxide using olive leaf extracts, study of its effect on some bacterial isolates under study, and study of the antioxidant activity of plant extracts.

2. MATERIALS AND METHODS

2.1 Subject

One hundred samples of (Dental Plaque) that accumulate on the surfaces of the teeth of children suffering from caries in their front or back baby teeth, whose ages ranged between 3 to 12 years, were collected during the period between September 2023 and March 2024 from the private dental clinic in the outpatient clinics. Dentists examined the children's teeth to determine caries and the appropriate place to take the swab for each sample separately. All children's data were recorded, including age, gender, chronic diseases, and not taking antibiotics in the past 24 hours. Samples were taken using a sterilized cotton swab and transferred by a media swab to conduct biochemical and diagnostic tests.

2.2 Isolation and diagnosis:

Gram-positive and Gram-negative bacteria were isolated from carious teeth of sick children according to the method (19, 20).

2.3 Antibiotic sensitivity testing:

The disk diffusion method was followed based on Bauer and his group's standard method using ready-made paper disks. Six antibiotics were used for this purpose: Cefepime 30 µg/disc, Gentamicin 10 µg/disc, Ampicillin 10 µg/disc, Tetracycline, and Methicillin. The bacterial suspension was prepared from the bacterial isolates under study by transferring 3-5 single colonies at 24 hours of age to 5 ml of standard saline solution. Then, the turbidity of the bacterial suspension was compared with the turbidity of the standard turbidity constant solution McFarland Solution, which gives an approximate number of cells equal to (10^8×1.5). - Spread the bacterial suspension using a glass diffuser of 0.1 ml using a cotton swab on Muller Hinton agar medium, then leave the plates for 15 minutes at room temperature until the bacterial culture dries. Antibiotic disks were transferred to the surface of the culture medium using sterile forceps at a rate of 6 disks per dish and then incubated for 24-48 hours at 37°C. The zone of inhibition for each tablet was measured and compared with the WHO standard tables, as reported in (21).

2.4 Antimicrobial activity test

The diffusion method was used to observe the effect of plant extracts on the growth of the isolated bacteria under study. The bacterial isolates were first grown in nutrient broth for 12-18 hours before use and standardized according to McFarland 0.5 standards. The solid Mueller Hinton agar medium was inoculated with sterile cotton from the bacterial suspension containing 10^8×1.5 cells/ml. Holes were made on the surface of the culture medium using a cork bore, and the prepared concentrations of each extract were placed at a rate of (0.1) ml for each hole. The dishes were left at room temperature for (20) minutes; then, the dishes were incubated at 37°C for 24 hours. The extract's effectiveness was determined by measuring the diameter of the inhibition zone around each hole in millimeters (22).

2.5 Preparation of olive leaf extract

The process involves collecting the plant and preparing fresh and healthy olive leaf extract. The leaves were collected from Salah Al-Din Governorate, and the fresh olive leaf extract was prepared using the green method, i.e., without using any toxic or dangerous chemical additives. We washed the collected leaves well with tap and deionized water to remove

surface contaminants and dust particles. The leaves were shade-dried at room temperature for 3 days. We prepared a powder from the dried leaves using an electric blender. 10.0 g of the powdered leaf was added to a 250 ml round-bottomed bottle containing 100 ml of deionized water, and the reflux process was carried out for 60 min at 80 °C. After cooling, we centrifuged the mixture at 10,000 rpm for 20 min to separate the extract from impurities. We stored the final extract in the refrigerator for further characterization or research(23).

(d) (c) (b) (a)

Figure 1: (a): shows (b) olive leaves, (c) olive powder, (d) olive leaf extract after centrifugation.

2.6 Study of the effect of plant extracts on bacterial growth:

The diffusion method was used to observe the effect of plant extracts on the growth of the isolated bacteria under study. The bacterial isolates were first grown in nutrient broth for 12-18 hours before use and standardized according to McFarland 0.5 standards. The solid Muller Hinton agar medium was inoculated with sterile cotton from the bacterial suspension containing 10^8×1.5 cells/ml. Holes were made on the surface of the culture medium using a cork bore, and the prepared concentrations of each extract were placed at a rate of (0.1) ml for each hole. The dishes were left at room temperature for (20) minutes and then incubated at 37°C for 24 hours. The extract's effectiveness was determined by measuring the diameter of the inhibition zone around each hole in millimeters(23).

2.7 Study of the inhibitory activity of calcium oxide nanoparticles:

2.7.1 Measurement method

The biological efficacy was measured according to (24) as follows:

1- Prepare the Mueller Hinton agar culture medium and pour the media after sterilizing it in an autoclave.
2- Prepare the bacterial vaccine for each bacterial sample separately at a concentration of (10^8 x 1.5) bacterial cells per milliliter, using sterile saline as a dilution solution and comparing it with the McFarland scale at a level of 0.5.
3- Using a cotton swab, a bacterial brush was created by spreading the bacterial inoculum over the entire culture medium.
4- Drilling holes after spreading the vaccine to load the nano-solutions into it with a volume of 100 microliters.
5- Incubating the medium in the laboratory incubator for 24 hours at a temperature of 37 degrees, after which the results are read by observing the presence or absence of inhibition around the holes loaded with nanomaterials.

2.7.2 Antioxidants activity

Control and reference solution: Negative control: DPPH reagent + DMSO.

Positive control or reference solution: ascorbic acid, Detector control: Detector without additives

2.7.2.1 Measurement method

The percentage of antioxidant activity (RSA) was measured according to(25-27) and is briefly as follows.

1- Prepare a DPPH solution and make a series of reference ascorbic acid concentrations.
2- Create a series of concentrations of the substance whose effectiveness is to be measured.
3- Add equal amounts of the purple DPPH indicator and the solution whose effectiveness is to be measured.
4- Leave the mixture in a dark environment for 30 minutes and observe the change in the color of the violet detector to yellow or transparent depending on the strength of the compound in preventing oxidation. Then the absorbance is measured with a spectrometer at 517 nm and then the value of the antioxidant activity is extracted according to the equation below.

$$\mathrm{RSA}(\%) = \left[\frac{(\mathrm{Ac} - \mathrm{As})}{\mathrm{Ac}}\right] \times 100 \text{ or } \mathrm{RSA}(\%) = \left[1 - \frac{\mathrm{Ac}}{\mathrm{As}}\right] \times 100$$

*Where Ac represents the absorbance value of the control and As represents the absorbance of the sample.

3. RESULTS AND DISCUSSION

3.1 Isolation and diagnosis:

The bacterial isolates were diagnosed based on the results of biochemical tests and the use of differential media. After diagnosis, 99 bacterial isolates of several genera were obtained, representing a percentage of 99.99%, as shown in Table (1).

Table (1): Numbers of isolated bacterial isolates and their percentages

	gram positive bacteria	Number of isolates	Percentage %
1	*Streptococcus mutans*	18	18.18
2	*Staphylococcus aureus*	16	16.16
3	*Micrococcus luteus*	16	16.16
4	*Enterococcus faecalis*	15	15.15
5	*Staphylococcus saprophyticus*	15	15.15
6	*Bacillus subtilius*	12	12.12
7	*Staphylococcus epidermis*	4	4.04
8	*Klepsiella pneumoniae*	3	3.03
9	Total number	99	99.99%

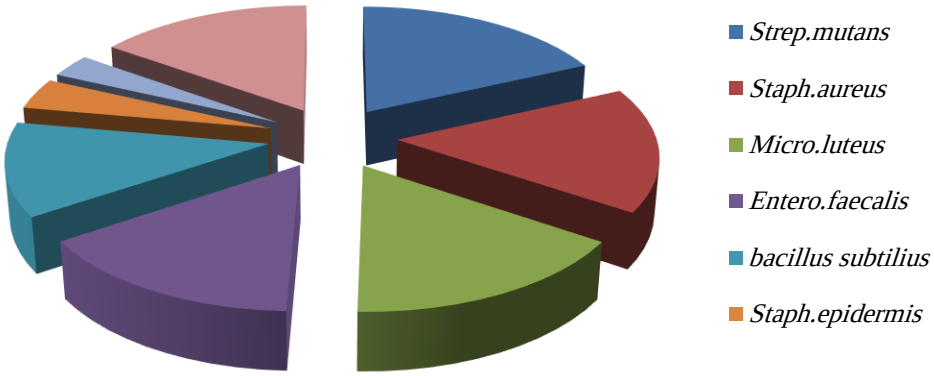


Figure (1) Types of bacteria causing tooth decay in children and their percentages.

The isolated *Streptococcus mutans* isolates were characterized by small spherical colonies. They were alpha-hemolytic when grown on Blood agar and appeared under the light microscope as Gram-positive spherules. Species of this genus gave negative tests for oxidase and catalase and were resistant to Bacitracin and Oxalic Acid. While Staphylococcus species appeared in irregular clusters, stained purple when stained with Gram stain, beta-hemolytic (i.e., partial hemolysis), positive for oxidase and catalase tests, as for the Coagulase test, all isolates of the bacterial genus Staphylococcus aureus showed a positive result. The Gram-negative isolates appeared as pink colonies and in single forms or short chains. *Klebsiella pneumonia* bacteria gave positive results for the VP and Simmon citrate tests while negative results for the MR and Indole tests.

3.2 Study the effect of antibiotics

Table (2) Resistance of bacterial isolates to antibiotics

Bacterial genus / **Antibiotics**	***Strep.mutans* (19)**	***Staph.aureus*(16)**	***K.pneumoniae*(3)**
	R %	R %	R %
Cefepime(KEP)	19 100 %	16 100 %	3 100 %
Chloramphenicol(C)	7 36.8 %	9 56.25 %	1 33.33 %
Gentamycin(CN)	4 21.1 %	2 12.5 %	2 66.66 %
Methicillin (ME)	16 84.2 %	15 93.75 %	2 66.66 %
Pencillin G (P)	17 89.6 %	15 93.75 %	3 100 %
Tetracycline (TE)	17 89.5 %	15 93.75 %	2 66.66 %

The results shown in the following table showed that the *S.mutans* isolates were 100% resistant to Cefepime, 85% to Pencillin and Tetracyclin, and 75% to Methicillin, while they were sensitive to Gentamycin and Chloramphenicol. This study differed from (Al-Samarrai, 2015), and these results partially agreed with the results reached by the researcher (Al-Mosawi, 2014). As for *S.aureus* bacteria, the results shown in the table showed that all *S.aureus* isolates were 100% resistant to the antibiotics Cefepime. This study agrees with the researcher (28)concluded, while 90% were resistant to Methicillin, Penicillin, and Tetracyclin antibiotics. It was 50% sensitive to the antibiotic Chloramphenicol, while the bacteria showed 90% sensitivity to Gentamicin. The results were consistent with the researcher (29) reached, and the result of this study differs from the researcher (30) reached. Finally, K. pneumoniae bacteria were 100% resistant to the antibiotic Cefepime Penicillin as a result of the excessive use of antibiotics, which led to mutations in the bacteria, as K. pneumoniae bacteria produce extended-spectrum β-lactamase, which increases the resistance of bacteria to antibiotics (31), and 70% to the antibiotics Gentamycin, Methicillin, Tetracyclin, and is consistent with Fouad *et al*., (32)reached, as the resistance rate to the antibiotic Gentamycin reached 67.5% and sensitivity to the antibiotic Chloramphenicol reached 70%.

3.3 Study of the inhibitory effectiveness of nano calcium oxide with plant extract

The results shown in Table (3) showed the effect of calcium oxide nanoparticles on the bacterial isolates under study isolated from dental caries infections in children. The plant extract of olive leaves recorded effectiveness against the bacterial genus Staph as the inhibition diameter reached 23 mm at a 100 mg/ml concentration, aureus. At the same time, the aqueous extract did not record any results against the other bacteria under study. As for the nano calcium oxide solution, it recorded effectiveness against *Staph.aureus* bacteria with an inhibition diameter of 29 mm.

Table (3): Results of the effectiveness of the nano calcium oxide solution

	Bacterial inhibition diameter of calcium oxide CaO measured in mm		
Bacteria	Plant extract	Cao standard solution	Nano solution
Staphylococcus aureus (S.a)	23	0	29
Streptococcus mutans (S.m)	0	0	0
Bacillus subtilius (B.s)	0	0	0
Micrococcus luteus (M.l)	0	0	0

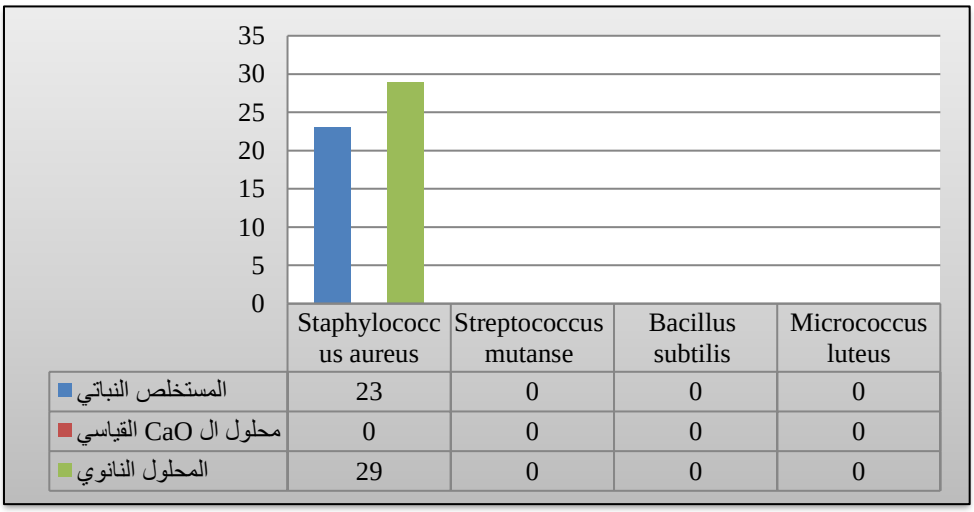


Figure (2): A diagram showing the effectiveness of nano calcium oxide against some bacterial species.

3.4 Study of the antioxidant activity (ROS%) of fig and olive leaves:

Table 3 and Figure 4 comprehensively show the results related to the percentage of inhibition of DPPH free radical by the aqueous extracts of fig and olive leaves. These results are coupled with the inhibition percentages achieved by the powerful antioxidant ascorbic acid, which was used as a positive control in this study. The IC50 value is a critical measure inversely related to a compound's antioxidant potential. In current terms, it quantifies the amount of antioxidants required to reduce the concentration of free radicals by 50%. A lower IC50 value indicates a higher antioxidant capacity of the compound under investigation (33). The aqueous extract of olive leaves appears to be the most effective, showing a value of 60 at a concentration of 50 micrograms. In contrast, ascorbic acid has a significantly low IC50 value, which aligns with its strong anti-radical prowess. However, it is important to realize that comparing these results with those of other studies is presented inappropriately due to various factors affecting the antioxidant content, including the choice of solvent and the adoption of different extraction techniques (34).

Olive leaf extract showed significant antioxidant capacity. These results are consistent with those reported by (35), who confirmed that olive leaf extract may have a relatively strong DPPH radical scavenging capacity at low concentrations. The potent antioxidant activity of olive leaves can be attributed to their high total content of polyphenolics and flavonoids (36, 37). The antioxidant capacity of olive leaf extract may encourage its use in the formulation of health-promoting foods and/or other dietary supplements to improve their functions.

Table (4) Shows the results of the antioxidant activity test

Ascorbic acid	Olives	Figs	Concentration - in micrograms
54.08	80	60	100
38.77	65	48	75
31.12	60	38	50
20.91	35	30	25
18.36	25	20	10
10	10	21	5
5	4	5	1

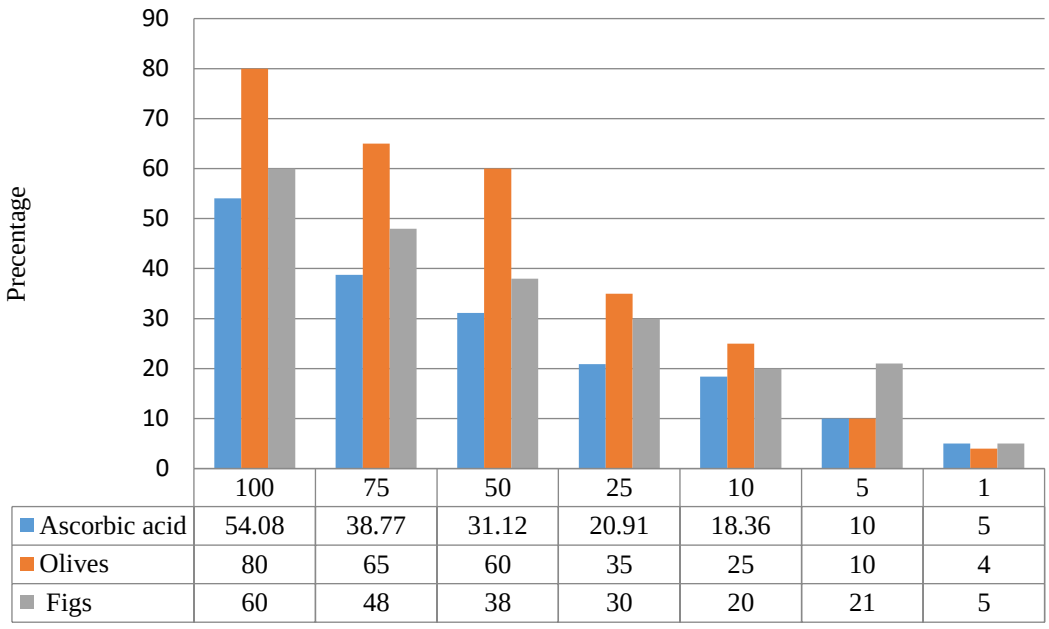
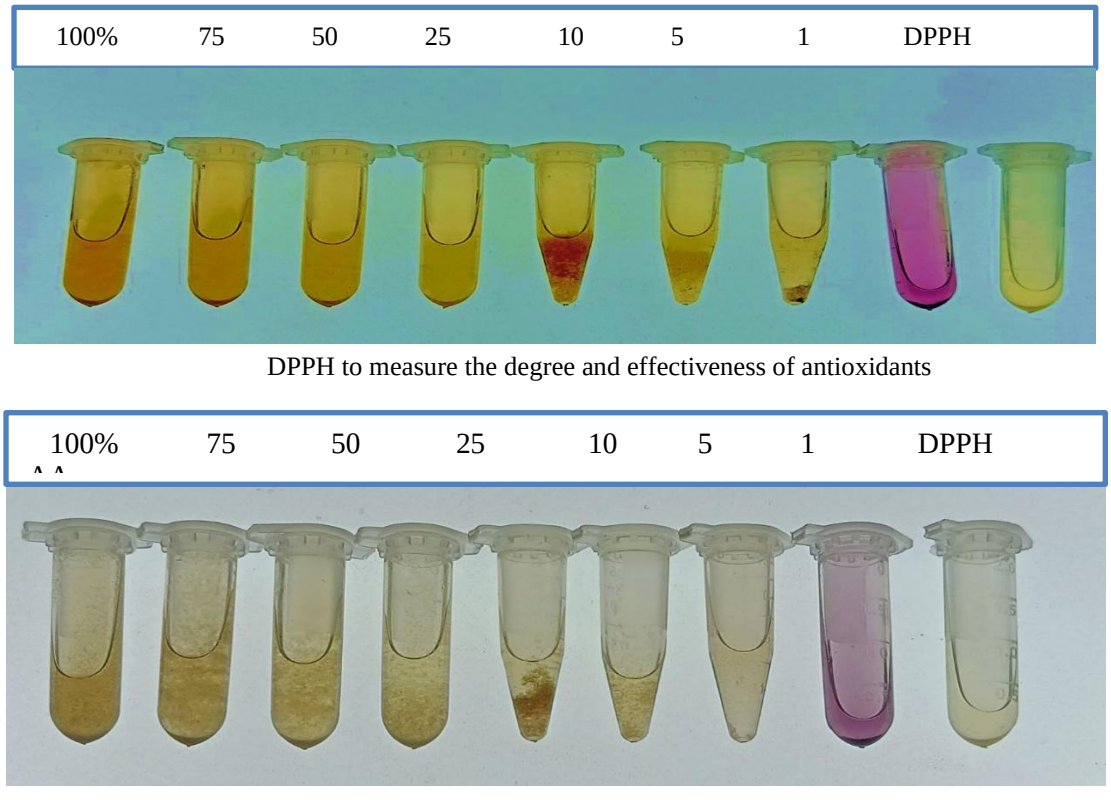


Figure 3: Anti-oxidant activity of ascorbic acid, olives and fig extracts

Figure (3), The percentage of the extracts' ability to remove free radicals (RSA) (antioxidant activity) AOs, where the horizontal axis represents the extract concentration and the vertical axis represents the percentage of effectiveness. We note that the effectiveness increases with increasing concentration and that all extracts were effective against free radicals.

Figure (4): MTP 96-well precision titration plate for antioxidant activity testing to measure the absorbance of the sample As and the absorbance of the control Ac.



DPPH to measure the degree and effectiveness of antioxidants

Figure (5): Shows the color change of the product of the reaction of fig extract with DPPH reagent to measure the degree and effectiveness of the antioxidant.

4. CONCLUSION

In summary, bacteria were isolated and identified from children with dental caries of both sexes and tested for their sensitivity to antibiotics. Biosynthesis of nano-calcium oxide from olive leaf extract was performed, and its inhibitory activity on some of the bacterial isolates under study was studied. Several bacterial genera cause dental caries in children, while *Strep. Mutans* constituted the most significant proportion. The results showed that most of the bacterial isolates under study show multiple resistance to many antibiotics. The current study concluded that olive leaf extract can manufacture calcium oxide nanoparticles. Tests proved they are nanoparticles with a spherical shape and less than 100 nm in size. In addition, olive leaf extracts have shown antibacterial effects superior to commercial pharmaceutical treatments. Therefore, olive leaves can be considered a potential antimicrobial agent, as they have a long history of use. However, further studies are needed to explore olive leaf extracts' phytochemical, molecular, and therapeutic properties to understand better the mechanisms that make these extracts effective as antibacterial agents.

REFERENCES

[1]. Martin-Kerry J, Gussy M, Gold L, Calache H, Boak R, Smith M, et al. Are Australian parents following feeding guidelines that will reduce their child's risk of dental caries? Child: Care, Health and Development. 2020;46(4):495-505. https://doi.org/10.1111/cch.12768

[2]. Amirhosseini MH, Kazemian H. Machine learning approach to personality type prediction based on the myers–briggs type indicator®. Multimodal Technologies and Interaction. 2020;4(1):9. https://doi.org/10.3390/mti4010009

[3]. Baker JL, Edlund A. Exploiting the oral microbiome to prevent tooth decay: has evolution already provided the best tools? Frontiers in microbiology. 2019;9:3323. https://doi.org/10.3389/fmicb.2018.03323

[4]. Long SS, Swenson R. Determinants of the developing oral flora in normal newborns. Applied and environmental microbiology. 1976;32(4):494-7. https://doi.org/10.1128/aem.32.4.494-497.1976

[5]. Shrivastava D, Srivastava KC, Dayakara JK, Sghaireen MG, Gudipaneni RK, Al-Johani K, et al. BactericidalActivity of Crevicular Polymorphonuclear Neutrophils in Chronic Periodontitis Patients and Healthy Subjects under the Influence of Areca Nut Extract: An In Vitro Study. Applied Sciences. 2020;10(14):5008. https://doi.org/10.3390/app10145008

[6]. Islam T, Islam MN, Zzaman W, Billah MM. Study of antimicrobial, antioxidant and cytotoxicity properties of selected plant extracts for food preservative applications. International Journal of Food Studies. 2021;10.

[7]. Bensid A, Ucar Y, Bendeddouche B, Özogul F. Effect of the icing with thyme, oregano and clove extracts on quality parameters of gutted and beheaded anchovy (Engraulis encrasicholus) during chilled storage. Food chemistry. 2014;145:681-6. https://doi.org/10.1016/j.foodchem.2013.08.106

[8]. Sudjana AN, D'Orazio C, Ryan V, Rasool N, Ng J, Islam N, et al. Antimicrobial activity of commercial Olea europaea (olive) leaf extract. International journal of antimicrobial agents. 2009;33(5):461-3. https://doi.org/10.1016/j.ijantimicag.2008.10.026

[9]. Huq MA, Ashrafudoulla M, Rahman MM, Balusamy SR, Akter S. Green synthesis and potential antibacterial applications of bioactive silver nanoparticles: A review. Polymers. 2022;14(4):742. https://doi.org/10.3390/polym14040742

[10]. Beyth N, Houri-Haddad Y, Domb A, Khan W, Hazan R. Alternative antimicrobial approach: nano-antimicrobial materials. Evidence-Based Complementary and Alternative Medicine. 2015;2015(1):246012. https://doi.org/10.1155/2015/246012

[11]. Zhang L, Pornpattananangkul D, Hu C-M, Huang C-M. Development of nanoparticles for antimicrobial drug delivery. Current medicinal chemistry. 2010;17(6):585-94. https://doi.org/10.2174/092986710790416290

[12]. Cavassin ED, de Figueiredo LFP, Otoch JP, Seckler MM, de Oliveira RA, Franco FF, et al. Comparison of methods to detect the in vitro activity of silver nanoparticles (AgNP) against multidrug resistant bacteria. Journal of nanobiotechnology. 2015;13:1-16. https://doi.org/10.1186/s12951-015-0120-6

[13]. Madhusudhana N, Yogendra K, Mahadevan K. A comparative study on Photocatalytic degradation of Violet GL2B azo dye using CaO and TiO2 nanoparticles. Int J Eng Res Appl. 2012;2(5):1300-7. DOI: 10.13140/RG.2.2.11328.17922
[14]. Roy A, Singh V, Sharma S, Ali D, Azad AK, Kumar G, et al. Antibacterial and dye degradation activity of green synthesized iron nanoparticles. Journal of Nanomaterials. 2022;2022(1):3636481. https://doi.org/10.1155/2022/3636481
[15]. AL-Jubori SH, Alsalman TH, Taqa AA. Evaluation of the Biocompatibility of a Newly Prepared Endodontic Biosealer using Subcutaneous Implant on Rabbits. International Journal of Enhanced Research in Medicines & Dental Care. 2017;4(6):5-14.
[16]. Abraham S, Sarathy V. Biomedical applications of calcium oxide nanoparticles-a spectroscopic study. Int J Pharm Sci Rev Res. 2018;49(1):121.
[17]. Sawai J. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. Journal of microbiological methods. 2003;54(2):177-82. https://doi.org/10.1016/S0167-7012(03)00037-X
[18]. MEDDOUR Elmoundir SM. Contribution à l'étude phytochimique et l'évaluation des propriétés antioxydante d'Olea europaea L 2021.
[19]. Cruickshank R, Duguid J, Marmion B, Swain R. Medical microbiology: the pratice of medical microbiology. Medical microbiology: the pratice of medical microbiology1975. p. 587-.
[20]. Collee JG, Fraser AG, Marmion BP, Simmons A. Practical medical microbiology1996.
[21]. Clsi C. M100-ED29: 2021 Performance Standards for Antimicrobial Susceptibility Testing. Clsi. 2020;40:50-1.
[22]. Abdulkareem EA, Abdulsattar JO, Abdulsattar BO. Iron (II) determination in lipstick samples using spectrophotometric and microfluidic paper-based analytical device (μPADs) platform via complexation reaction with iron chelator 1, 10-phenanthroline: A comparative study. Baghdad Science Journal. 2022;19(2):0355-.
[23]. Alaallah NJ, Abd Alkareem E, Ghaidan A, Imran NA. Eco-friendly approach for silver nanoparticles synthesis from lemon extract and their anti-oxidant, anti-bacterial, and anti-cancer activities. Journal of the Turkish Chemical Society Section A: Chemistry. 2023;10(1):205-16. https://doi.org/10.18596/jotcsa.1159851
[24]. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis. 2016;6(2):71-9. https://doi.org/10.1016/j.jpha.2015.11.005
[25]. Thi Pham TN, Nguyen TT, Le Thi Nguyen T, Nguyen Tran AM, Nguyen TN, Tong DT, et al. Antioxidant and Anti-Inflammatory Activities of Phytochemicals from Ruellia tuberosa. Journal of Chemistry. 2022;2022(1):4644641. https://doi.org/10.1155/2022/4644641
[26]. Gulcin İ, Alwasel SH. DPPH radical scavenging assay. Processes. 2023;11(8):2248. https://doi.org/10.3390/pr11082248
[27]. Martínez-Cabanas M, López-García M, Rodríguez-Barro P, Vilariño T, Lodeiro P, Herrero R, et al. Antioxidant capacity assessment of plant extracts for green synthesis of nanoparticles. Nanomaterials. 2021;11(7):1679. https://doi.org/10.3390/nano11071679
[28]. Mustafa YF, Al-dabbagh KA, Mohammed MF. Synthesis of new metronidazole derivatives with suspected antimicrobial activity. Iraqi Journal of Pharmacy. 2008;7(1):34-41.
[29]. Hasan SA, Saleh I, Ali H. Bacteriological and Molecular Detection of Staphylococcus Aureus and its Resistance to Methicillin among Specimens from Kirkuk Community. Annals of the Romanian Society for Cell Biology. 2021;25(7):461-73.
[30]. NNENNA MC. METHICILLIN AND VANCOMYCIN RESISTANT STAPHYLOCOCCUS AUREUS COLONIZATION OF MEDICAL EQUIPMENTS AND SURFACES WITHIIN TERTIARY HEALTH CARE INSTITUTIONS IN SUB-SAHARAN AFRICA (A CASE STUDY): UNIVERSITY OF IBADAN; 2019.
[31]. Wei Z-Q, Chen Y-G, Yu Y-S, Lu W-X, Li L-J. Nosocomial spread of multi-resistant Klebsiella pneumoniae containing a plasmid encoding multiple β-lactamases. Journal of medical microbiology. 2005;54(9):885-8. https://doi.org/10.1099/jmm.0.46151-0
[32]. Fouad EA, Elnaga ASA, Kandil MM. Antibacterial efficacy of Moringa oleifera leaf extract against pyogenic bacteria isolated from a dromedary camel (Camelus dromedarius) abscess. Veterinary World. 2019;12(6):802. https://doi.org/10.14202/vetworld.2019.802-808
[33]. Ouryemchi I, Oubihi A, Taibi M, Elbouzidi A, Jaber H, Haida S, et al. Phytochemical Components, Antioxidant Properties, Antimicrobial Effects, and Bio-Aphicidal Prospects Against the Black Bean Aphid (Aphis fabae Scop.) of Olea europaea L. Leaves Extracts from Morocco. Journal of Ecological Engineering. 2024;25(3). https://doi.org/10.12911/22998993/177639
[34]. Oubihi A, Hosni H, Nounah I, Ettouil A, Harhar H, Alaoui K, et al. Phenolic Content, Antioxidant Activity, Anti-Inflammatory Potential, and Acute Toxicity Study of Thymus leptobotrys Murb. Extracts. Biochemistry Research International. 2020;2020(1):8823209. https://doi.org/10.1155/2020/8823209
[35]. Saija A, Trombetta D, Tomaino A, Cascio RL, Princi P, Uccella N, et al. In vitro'evaluation of the antioxidant activity and biomembrane interaction of the plant phenols oleuropein and hydroxytyrosol. International Journal of Pharmaceutics. 1998;166(2):123-33. https://doi.org/10.1016/S0378-5173(98)00018-0

[36]. Bouaziz M, Fki I, Jemai H, Ayadi M, Sayadi S. Effect of storage on refined and husk olive oils composition: Stabilization by addition of natural antioxidants from Chemlali olive leaves. Food Chemistry. 2008;108(1):253-62. https://doi.org/10.1016/j.foodchem.2007.10.074
[37]. Salah MB, Abdelmelek H, Abderraba M. Study of phenolic composition and biological activities assessment of olive leaves from different varieties grown in Tunisia. Med chem. 2012;2(5):107-11. http://dx.doi.org/10.4172/2161-0444.1000124